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Chemically Controlled Amplified Ratiometric Fluorescence in Surface-Immobilized End-Capped Oligo(*p*-phenylene ethynylene)s

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Efficient transport of excited states in the extended π-electron conjugated molecular systems makes them especially valuable as a basis for designing chemosensing and optoelectronic devices.[1] Chemosensory devices greatly benefit from signal amplification resulting from the facility of this process.[2] In the case of isolated molecules (e.g., in dilute solutions) the intramolecular energy migration dominates both by through-space Förster and through-bond Dexter mechanisms,[3] although it is often considered to be not very efficient.[4] If a higher energy fluorophore (donor) is chemically linked to a lower energy fluorophore (acceptor), and the electronic interactions between these two groups are relatively weak, the resulting dyad may exhibit dual fluorescent emission upon exciting the higher energy fluorophore. Furthermore, the relative ratio of the two emission bands can be controlled by attenuation of the energy transfer (ET) within the dyad (ET cassette).[5] This principle has been previously explored in the design of ratiometric fluorescent sensors, where ET between the donor and acceptor units was controlled either by changing the effective spatial separation between the fluorophores[6] or by chemical modification of one of the two units.[7]

Compared to intramolecular ET in dilute solutions, much higher efficiency can be achieved in the molecular aggregates and in solid films where the exciton migration occurs as a three-dimensional process both by inter- and intramolecular pathways.[8] In such systems, the energy migration becomes particularly sensitive to the factors capable of influencing its efficiency such as small electronic perturbations of the donor and/or acceptor units. We are particularly interested in studies of ET in monodisperse oligo(*p*-phenylene ethynylene)s (OPEs) terminated with a lower energy gap group and covalently immobilized on a surface to yield monolayer thin films. Upon achieving the uniform molecular arrangement, these systems display a very efficient ET. Remarkably, this ET process is extremely sensitive to chemical modifications of the film, producing a dramatically enhanced ratiometric fluorescent response on such events. Here, we report a first example of this unprecedented phenomenon.

The 10-formylanthracenyl-terminated OPE **1** (Figures 1A and 2) was chosen since anthracene derivatives are very convenient low energy gap units for ET studies in (*p*-phenylene ethynylene) systems.[9] Immobilization of **1** on glass slides occurred smoothly from its solution in toluene, and afforded the monolayer film with a relatively uniform surface coverage.[10] The surface density (estimated from electrochemical studies on the monolayer film immobilized on ITO/glass surface) was 2.5×10^{-11} mol cm^{-2}, which corresponds to the occupied area of 7 nm^2 per molecule. With the theoretical estimate ranging from 2 to 4 nm^2 per molecule, this indicated a relatively densely packed film. Absorption spectra of **2** in $CHCl_3$ solution and immobilized monolayer of **1** possess similar features, with the major band at ~380 nm from the OPE core, and a much less intense band at ~450 nm corresponding to the anthracenyl unit (Figure S1 in Supporting Information). In

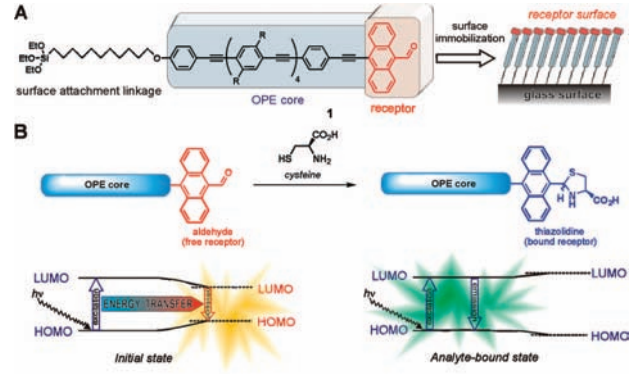

Figure 1. (A) General structure of end-functionalized OPE **1** and its immobilization on glass surface; (B) reaction of **1** with cysteine and a schematic diagram to show origin of the ratiometric fluorescent response.

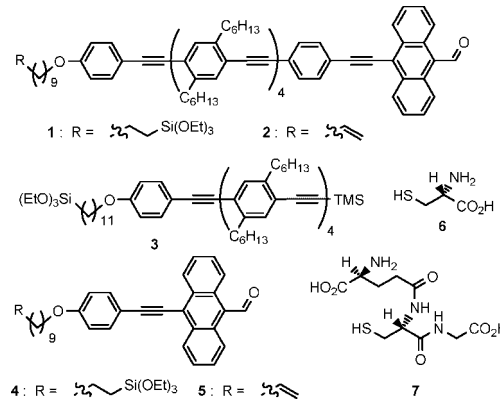

Figure 2. Structures of OPE **1**, reference compounds **2**−**5**, L-cysteine **6**, and L-glutathione **7**.

fluorescence spectrum in dilute solution, the most intense band with a maximum at 416 nm corresponds to emission from the OPE core. Relatively inefficient intramolecular ET from the OPE core to the anthracenyl acceptor leads to appearance of the less intense broad band at 520 nm (Figure 3A). While not efficient in solution, the ET process becomes highly efficient in the densely packed organized monolayer film, where it results in the dominating anthracenyl emission, with only a small residual band of the OPE core. That the 520 nm main emission band in the film indeed originated from the anthracenyl group, and not produced from intermolecular interactions of the closely packed OPE moieties (or aggregation-related excimer formation),[11] was confirmed by the presence of only a single OPE band at 430 nm in the fluorescence spectrum of the immobilized monolayer of reference compound **3** lacking the anthracenyl group (Figure 3A).

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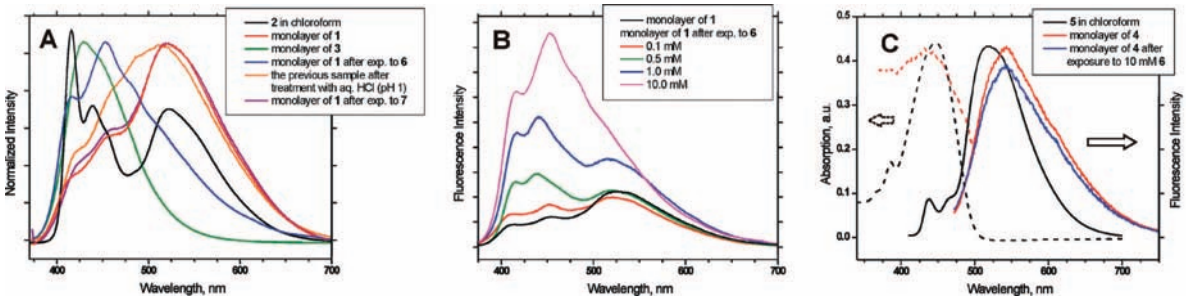

Figure 3. (A) Normalized fluorescence spectra of **2** in $CHCl_3$ (fluorescence quantum yield 0.08), monolayer of **3**, and monolayer of **1** before and after exposure to aq **6** (10 mM) and **7** (20 mM), as well as recovery of the initial fluorescence pattern after treatment of the monolayer pre-exposed to **6** with aq HCl (pH 1); (B) change in fluorescence of the monolayer of **1** upon exposure to increasing concentrations of **6**; (C) absorption (dash line) and fluorescence (solid line) spectra of **5** in $CHCl_3$ (extinction coefficient ε(432 nm) 1.13×10^4, fluorescence quantum yield 0.02), and excitation (dash line) and fluorescence (solid lines) spectra of monolayer of **4** before and after exposure to aq **6** (10 mM).

Exposure of a glass slide modified with monolayer of **1** to a 10 mM aqueous buffered solution of L-cysteine **6** led to significant diminishing of the 520 nm anthracenyl emission band and simultaneous growth of the OPE band with a maximum at 450 nm (Figure 3A). This ratiometric behavior follows the principle schematically outlined in Figure 1B. Reaction with cysteine results in aldehyde to thiazolidine conversion, which perturbs the electronic structure of the anthracenyl receptor terminus resulting in diminished ET from the OPE core to the end group, with concomitant intensity increase of the OPE emission band. Thus, the system clearly exhibits the expected ratiometric behavior.

A more systematic study revealed a gradual intensity increase of the 450 nm emission band upon exposure of the monolayer to increasing concentrations of **6** (Figure 3B). The observed intensity increase was significant for cysteine concentrations from 0.1 to 10 mM, and resulted in a 3-fold total enhancement of the fluorescence quantum yield, with the 450 nm band being the major contributor. This increase was in agreement with much higher fluorescence quantum yield of the OPE vs the anthracenyl fluorophore (0.41 for **3** vs 0.02 for **5**). While the OPE emission band showed monotonic intensity increase, the behavior of the anthracenyl band at 520 nm was more complex. Exposure of the monolayer to low concentrations of **6** resulted in the expected, albeit small, decrease of the anthracenyl band intensity. However, with increasing concentration of **6**, the intensity started increasing again, eventually surpassing its initial value in the pristine monolayer. This can be understood by considering the significant enhancement of the OPE emission upon blocking intramolecular ET to the acceptor fluorophore. This enabled delivering (via intermolecular pathway) more excitation energy to the unreacted molecules of **1** surrounding cysteine-bound **1** in the monolayer film, therefore enhancing their anthracenyl emission.

This observation necessarily implies that even upon exposure to high cysteine concentrations the majority of aldehyde groups in the monolayer remained intact, and only a small fraction of them reacted on the film surface. Indeed, in a control experiment, a surface-immobilized monolayer of the anthracenyl receptor **4** showed only a subtle fluorescent response on a prolonged exposure to aqueous cysteine solution (Figure 3C). The lack of response is likely due to the unfavorable reaction equilibrium of the anthraldehyde receptor toward thiazolidine formation.[10] That the formation of the "elusive" thiazolidine was the actual reason for the observed ratiometric response was evidenced by the finding that exposure of the monolayer of **1** to a cysteine-free buffer or to an aqueous solution of the closely related (but not capable of forming a thiazolidine) glutathione **7** did not produce any fluorescence change (Figure 3A). Experimentally observed recovery of the initial fluorescence pattern after treatment of the cysteine-exposed monolayer of **1** with dilute HCl (Figure 3A) likely stems from hydrolysis of the surface thiazolidine groups. Also, trace thiazolidine formation in the monolayer was found in the XPS experiments (Figure S2 in Supporting Information).[10]

The observation that the minor extent of the chemical conversion in the surface-immobilized monolayer of OPE **1** could trigger a significant ratiometric fluorescent response is without precedent. Although more studies are required to completely understand this unusual "turn-on" amplification, the improved molecular organization in the monolayer likely plays a role in it. On the practical side, this phenomenon can provide a useful platform to increase optical gain in fluorescent chemodetection.

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Supporting Information Available: Detailed synthetic and experimental procedures and additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Thomas, S. W., III.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339–1386.
(2) (a) Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201–207. (b) Chen, L.; McBranch, D. W.; Wang, H.-L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 12287–12292.
(3) (a) TurroN. J. *Modern Molecular Photochemistry;* University Science Books: Mill Valley, CA, 1991; (b) Dias, F. B.; Knaapila, M.; Monkman, A. P.; Burrows, H. D. *Macromolecules* **2006**, *39*, 1598–1606.
(4) (a) Beljonne, D.; Pourtois, G.; Silva, C.; Hennebicq, E.; Hertz, L. M.; Friend, R. H.; Scholes, G. D.; Setayesh, S.; Müllen, K.; Brédas, J. L. *Proc. Nat. Acad. Sci. U.S.A.* **2002**, *99*, 10982–10987. (b) Nguyen, T.-Q.; Wu, J.; Doan, V.; Schwartz, B. J.; Tolbert, S. H. *Science* **2000**, *288*, 652–656. (c) Becker, K.; Lupton, J. M.; Feldman, J.; Setayesh, S.; Grimsdale, A. C.; Müllen, K. *J. Am. Chem. Soc.* **2006**, *128*, 680–681.
(5) (a) Kim, T. G.; Castro, J. C.; Loudet, A.; Jiao, J. G.-S.; Hochstrasser, R. M.; Burgess, K.; Topp, M. R. *J. Phys. Chem. A* **2006**, *110*, 20–27. (b) Loudet, A.; Bandichhor, R.; Wu, L.; Burgess, K. *Tetrahedron* **2008**, *64*, 3642–3654.
(6) Kim, H. J.; Park, S. Y.; Yoon, S.; Kim, J. S. *Tetrahedron* **2008**, *64*, 1294–1300.
(7) (a) Takakusa, H.; Kikuchi, K.; Urano, Y.; Kojima, H.; Nagano, T. *Chem.—Eur. J.* **2003**, *9*, 1479–1485. (b) Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc.* **2006**, *128*, 14474–14475. (c) Tremblay, M. S.; Halim, M.; Sames, D. *J. Am. Chem. Soc.* **2007**, *129*, 7570–7577.
(8) Schwartz, B. J. *Annu. Rev. Phys. Chem.* **2003**, *54*, 141–172.
(9) (a) Swager, T. M.; Gil, C. J.; Wrighton, M. S. *J. Phys. Chem.* **1995**, *99*, 4886–4893. (b) Nesterov, E. E.; Zhu, Z.; Swager, T. M. *J. Am. Chem. Soc.* **2005**, *127*, 10083–10088.
(10) See Supporting Information for experimental details and analytical data.
(11) (a) Halkyard, C. E.; Rampey, M. E.; Kloppenburg, L.; Studer-Martinez, S. L.; Bunz, U. H. F. *Macromolecules* **1998**, *31*, 8655–8659. (b) Kim, Y.; Bouffard, J.; Kooi, S. E.; Swager, T. M. *J. Am. Chem. Soc.* **2005**, *127*, 13726–13731. (c) Li, H.; Powell, D. R.; Hayashi, R. K.; West, R. *Macromolecules* **1998**, *31*, 52–58.

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